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SELECTIVE RESPONSIVENESS OF POLYMODAL NOCICEPTORS OF THE RABBIT EAR TO CAPSAICIN, BRADYKININ AND ULTRA-VIOLET IRRADIATION

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SUMMARY

1. The activity of single C- and A-fibre cutaneous sensory units was recorded from the great auricular nerve of anaesthetized rabbits to compare the effects of chemical with other forms of stimulation under several experimental conditions. Chemical agents were delivered by close arterial injection.

2. Small intra-arterial injections of bradykinin (0·2 μg) and a substantial range of capsaicin doses (2–200 μg) consistently activated C polymodal nociceptors without exciting other types of C- or A-fibre cutaneous sense organs.

3. Topical application of xylene to the receptive field of polymodal nociceptors evoked a strong excitation which lasted several minutes.

4. The responses of polymodal nociceptors to mechanical, chemical (bradykinin, xylene) and noxious thermal stimuli were suppressed or abolished after large intra-arterial doses of capsaicin. Capsaicin desensitization of polymodal nociceptors to one kind of stimulation often was not paralleled by similar changes in responsiveness to other stimuli. However, on the average, capsaicin desensitization altered responses to thermal, chemical and mechanical stimuli without afferent selectivity.

5. Background discharge developed in C polymodal nociceptors of the rabbit ear following ultra-violet irradiation sufficient to produce evidence of delayed inflammation. Noxious heat and bradykinin injection (0·2 μg) evoked more activity from C polymodal nociceptors in the irradiated ears than from control units.

INTRODUCTION

The responsiveness of cutaneous sense organs to mechanical and thermal stimuli has been studied in some detail and is the basis of their current classification. Much less is known about the effects of chemical agents upon the excitability of such sensory units, although the skin is constantly exposed to a changing chemical environment on both its external surface and its internal boundary. Furthermore, chemical substances contacting the skin surface and cutaneous inflammatory processes, which involve complex chemical events, evoke sensory experiences including pain and hyperalgesia.

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One type of cutaneous sensory unit, the C polymodal nociceptor, is excited by noxious chemical agents applied topically to intact skin (Bessou & Perl, 1969). Chemical substances applied to the base of a burn blister are reported to activate only nociceptors (Foster & Ramage, 1981). With the apparent exception of some warm units, other kinds of cutaneous sensory units are not excited by applying chemicals to the skin surface (Foster & Ramage, 1981; Kenins, 1982; Szolcsányi, 1982). On the other hand, close arterial injection of pain-producing substances such as bradykinin excites other cutaneous sensory units in addition to C-fibre polymodal nociceptors (Beck & Handwerker, 1974; Handwerker, 1977). These findings imply that chemical action on sense organs of the skin could be selective by one mode of administration but not by another and as a consequence may be relevant to mechanisms operating in cutaneous pain.

Capsaicin, the irritant ingredient of red pepper, has been proposed as a tool for studying mechanisms of chemogenic pain (Jancsó, Jancsó-Gábor & Szolcsányi, 1967; Jancsó, 1968), and in particular for determining the functional role of polymodal nociceptors (Szolcsányi, 1977). Capsaicin, like other irritants, excites polymodal nociceptors when applied topically to the blister base of the cat's leg (Foster & Ramage, 1981) or to the intact skin of the rat (Kenins, 1982) and human beings (Konietzny & Hensel, 1983). However, the selectiveness of this agent for close arterial injection among different types of cutaneous sensory units has not been established.

A long-lasting irreversible loss of responsiveness to noxious chemical stimuli takes place in a variety of animals when capsaicin is applied in high concentrations topically, on nerve fibres, or intrathecally. When rodents (rat, mouse and guinea-pig) are pre-treated by systemic injections of capsaicin a similar desensitization is noted. However, the reports of the reactions of such desensitized animals to noxious mechanical and thermal stimuli have not been consistent, particularly in the case of rats pre-treated neonatally (for reference see Szolcsányi, 1982, 1985; Nagy, 1982; Fitzgerald, 1983; Russell & Burchiel, 1984). Recent single-unit studies have shown that long-term functional impairment after applying capsaicin to axons occurs only for polymodal nociceptors, while systemic pre-treatment of neonatal rats results in an indiscriminate loss of afferent C fibres (Lynn, Carpenter & Pini, 1984; Handwerker, Holzer-Petsche, Heym & Welk, 1984; Welk, Fleischer, Petsche & Handwerker, 1984).

The present work was aimed at answering some of these unsettled issues on the chemical responsiveness of cutaneous sensory units. The effects of intra-arterial injection of bradykinin and capsaicin and topical application of xylene were examined on the different kinds of cutaneous sensory units common in the rabbit's ear. Also described are changes in responsiveness of C-fibre polymodal nociceptors after acute capsaicin desensitization and in the early stage of inflammation evoked by ultra-violet irradiation of the ear. Preliminary reports of the results have been communicated (Szolcsányi, 1980).

METHODS

Results are reported from experiments on fifty-seven domestic rabbits (*Oryctolagus cuniculus*) deeply anaesthetized with intravenous pentobarbitone (40 mg/kg), supplemented as required to maintain areflexia during the surgical procedures. Mean arterial pressure with this anaesthetic

schedule remained about 100 mmHg. Before the electrophysiological recordings were started the animal was paralysed with gallamine (Flaxedil) and placed on artificial respiration. The arterial pressure was then used to judge the need for supplemental anaesthesia, enough anaesthetic being given to maintain the blood pressure at the pre-paralysis level. Rectal and ear temperatures were monitored using thermistors and were maintained between 37 and 38 °C using a heating pad and a heat lamp. The left ear was shaved and fixed to a supporting plate with modelling clay. The great auricular nerve and artery were exposed at the base of the ear, and a polyamide cannula for injecting chemical solutions was inserted into a small branch of the artery near the dorsum of the ear. The great auricular nerve was cut at its junction in the neck, and fine filaments were dissected and placed across a platinum wire electrode for recording. Standard techniques for single-unit recording were used as previously described (Bessou & Perl, 1969; Shea & Perl, 1985). The effectiveness of isolating a single unit was tested by recording the response to supramaximal shocks delivered by electrodes located at the distal part of the exposed nerve. In five animals, recordings were made from myelinated fibres in the nerve trunk with high-impedence (30–80 MΩ) micropipettes filled with 2 M-potassium acetate (Burgess & Perl, 1967). Analogue magnetic-tape recordings of the discharges and parallel records of stimulus parameters were digitized. Analyses of the quantitative discharge were performed using off-line programs of the type described by Schmittroth in Bessou & Perl (1969) for a digital computer-interactive graphics system.

Thermal, mechanical and chemical stimulation

A programmable contact thermal stimulator was used, the temperature of which was regulated by a servo loop from a small thermistor fixed to the silver tip of the thermode. The tip was gently pressed against the skin firmly contacting an area of approximately 3 mm^2. Stepwise heating or cooling started in most cases from 30 °C. Each step lasted 17 s and the temperature difference between consecutive steps was 3 °C.

Threshold response to mechanical stimulation was determined using a series of nylon monofilaments bending at forces ranging from 4·5 mg to 16 g. The receptors were classified according to schema suggested by Perl & Burgess (1973).

Chemical agents, diluted in 0·2 ml of 0·9 % NaCl, were injected into the side branch of the great auricular artery for a period of 5–7 s while the main vessel was clamped central to the injection site. A 1 % (w/v) stock solution of capsaicin was prepared using ethanol and Tween 80, as described earlier (Jancsó *et al.* 1967). Further dilution were made with 0·9 % NaCl. Intra-arterial chemical stimulation was tested only on those units with a receptive field within the region of distribution of the cannulated artery. This was established prior to use of chemical stimulants by injecting Evans Blue (200 μg in 0·2 ml) into the bloodstream. The effects of xylene applied topically to the receptive field or those of capsaicin injected (in 0·1 ml) into the receptive field, or both, were examined in some units.

Ultra-violet irradiation of the ear

One ear from each of fourteen rabbits was irradiated by ultra-violet (U.V.) light to examine the responsiveness of cutaneous sensory units in the early stages of inflammatory reaction.

Under pentobarbitone anaesthesia, the ear (supported as described earlier) was irradiated for 4 h using a small U.V. lamp placed 3–4 cm above its outer surface. The surface temperature of the ear, measured with a contact thermistor, remained under 39·5 °C. During the period of irradiation only slight hyperemia developed. In two control experiments, pronounced hyperemia with slight edema became evident 1 day later. In order to reveal changes at times when the tissue response to the radiation was minimal, the auricular nerve was exposed immediately after irradiation was complete, and all C-fibre units whose discharges could be isolated were tested by chemical, thermal and mechanical stimuli.

Statistics

The possibility of chance differences in the number of discharges for a given unit was calculated using the Wilcoxon matched-pairs signed-rank test. The means of two groups of units were compared using the Wilcoxon rank sum test (Remington & Schork, 1970).

RESULTS

Afferent units studied in intact skin

The responsiveness of ninety-six afferent units was studied. A high proportion of Aδ or C afferent fibres was selected, since nociceptors are contained in these groups of units. Only two specific cold receptors and some down hair (D-hair) receptors situated near pulsatile vessels exhibited spontaneous activity. The solvent alone placed on the receptive field elicited discharge in two low-threshold C mechanoreceptors and one D-hair unit. In these three cases the number of impulses elicited by control injections was deducted from the response. Each testing was followed by a pause of at least 15 min, and noxious heating was applied only after the first injections.

Polymodal nociceptors

A total of thirty-six polymodal nociceptors with afferent fibres conducting at $0{\cdot}94 \pm 0{\cdot}47$ (S.D.) m/s were chemically tested. Their mechanical thresholds were $14{\cdot}1 \pm 6{\cdot}89$ (S.D.) g/mm^2, and thirty-four (94%) had a threshold between 7·7 and 28 g/mm^2. Presuming the same nylon filament sizes were used, these figures may be somewhat lower than those obtained on the polymodal nociceptors of the rabbit saphenous nerve (Lynn, 1979). The threshold for stepwise temperature increases was below 54 °C for all such units. After preliminary experiments, this value was set as the upper heating limit to minimize severe tissue damage. Heat response parameters were calculated using Lynn's method (1979) on fifteen units for which the appropriate data were available. The temperature required to produce an average firing of 1 s^{-1} (T_1) varied from 49·6 to 55·5 °C, and the increase in firing rate for a 10 °C rise in skin temperature (Q_{10}) was $39 \pm 5{\cdot}2$ (S.D.) ranging from 7·1 to 112·2. These values, including the large range of Q_{10} values, correspond well with those reported for polymodal nociceptors of the rabbit hind leg (Lynn, 1979).

Capsaicin (2 or 20 μg) and bradykinin (0·2 μg) injected intra-arterially consistently activated the twenty-nine polymodal nociceptors tested (Table 1). 20 μg of capsaicin initiated responses of short latency (1–3 s), short duration ($14{\cdot}3 \pm 1{\cdot}5$ s) and high instantaneous frequency; the average discharge and the peak instantaneous frequency were $35 \pm 5{\cdot}6$ and $26 \pm 3{\cdot}8$ impulses/s, respectively (mean ± S.E., $n = 15$). At 15 min intervals, 2 and 20 μg doses gave closely reproducible responses. Higher doses of capsaicin applied at shorter intervals evoked progressively smaller responses (Fig. 1).

Capsaicin was injected subcutaneously under the receptive fields of seven polymodal nociceptors whose fields were not served by the cannulated artery. Two of these seven units were excited by 1 μg, and the remainder by 10 μg. The solvent was either ineffective (four cases) or produced much less effect (three cases).

Fig. 1. Response of a C polymodal nociceptor of the rabbit ear to repeated intra-arterial injections of capsaicin. Number of discharges in each 2 s period (*A* and *B*) and in 1 s (*C*). The marks below the graphs indicate the duration of the capsaicin injection. Doses: 20 μg (*A*), 200 μg (*B*) and 600 μg (*C*). Note the reproducibility with the small dose and the desensitization after higher doses.

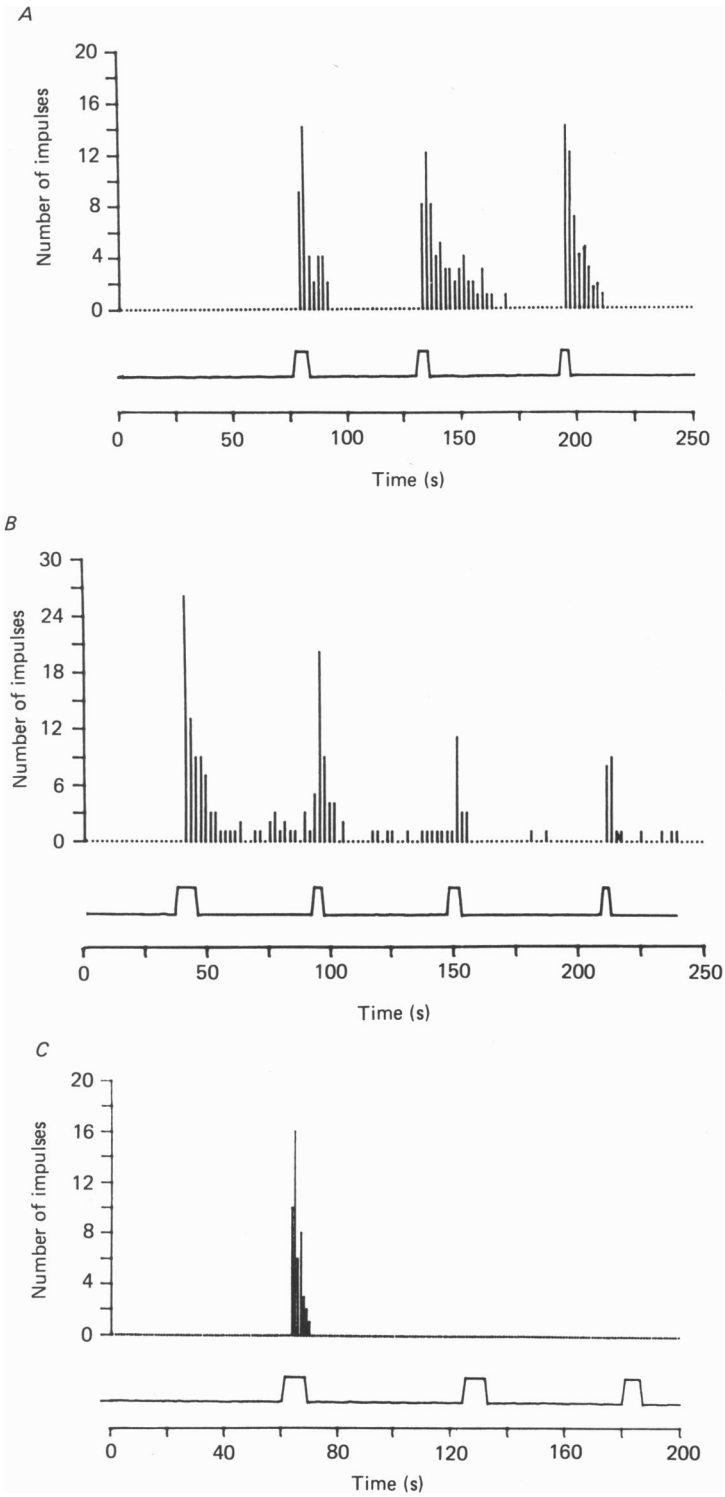

Fig. 1. For legend see opposite.

Responses to intra-arterial bradykinin had longer latencies than for capsaicin and were characterized by a discharge that persisted longer and decayed more slowly (Fig. 2). A small dose of bradykinin, 0·2 μg, consistently activated polymodal nociceptors (Table 1) and on the average evoked a similar number of discharges as 20 μg of capsaicin (40 $\pm$ 10·8 impulses; mean $\pm$ S.E.) from the same fifteen units. The bradykinin responses varied substantially in latency from 5 to 40 s (average 13·9 $\pm$ 2·8 s, mean $\pm$ S.E.) and in the duration of firing from 14 to 167 $\pm$ 41 s. Peak instantaneous frequency (9·73 $\pm$ 3·7 impulses/s) for the bradykinin usually appeared with 20–50 s. Injection of tenfold increased doses of bradykinin, 2 μg, excited polymodal nociceptors after a considerably shorter latency but caused circulatory disturbance of the ear, as indicated by a local cyanosis. Consequently the effect of these large doses was not analysed quantitatively.

TABLE 1. Responses of different sensory units to capsaicin and bradykinin

Receptor type	Fibre type	Conduction velocity (m/s)		Activated units/tested units				
				Capsaicin (μg)			Bradykinin (μg)	
		Mean	$\pm$S.D.	200	20	2	2	0·2
Polymodal nociceptor	C	0·94	0·15	14/14	25/26	9/16	7/7	22/23
High-threshold mechanoreceptor	C	1·09	0·18	0/2	0/4		1/4	0/4
Low-threshold mechanoreceptor	C	1·08	0·15	0/3	0/7		1/3	0/7
Cold receptor	C	0·80, 0·82		0/2	0/2			
High-threshold mechanoreceptor	Aδ	23·70	5·6	0/4	0/8			
D hair	Aδ	14·30	3·8	0/5	0/11		0/4	0/11
G hair	A$\alpha\beta$	36·60	14·7	0/5	0/12		0/3	0/6
Field	A$\alpha\beta$	33·20	4·2		0/3		0/1	0/3
Deep mechanical non-responsive	C	0·75, 0·81		2/2	2/2			1/1
Mechanical non-responsive	Aδ	16·50	22·4		0/2			0/1

Intra-arterial serotonin (5-HT) was tested on four polymodal units; 2 μg was ineffective and 20 μg, in three units only, initiated a much lower frequency of discharge than did capsaicin or bradykinin. Therefore this agent was not further evaluated.

One drop of xylene was applied to the surface of the receptive field of nine units. In every instance it evoked remarkable activity lasting over 5 min and, in four trials, over 10 min. The build-up to and decay from the sustained peak were gradual, but intermittent bursts of discharges appeared frequently at relatively high frequencies (Fig. 3). After the activity evoked by the xylene ceased, mechanical threshold was tested on four units and no change was evident.

Desensitization of polymodal nociceptors by capsaicin

The effects of capsaicin given intra-arterially in quantities large enough to produce desensitization were analysed by assessing the responsiveness of polymodal noci-

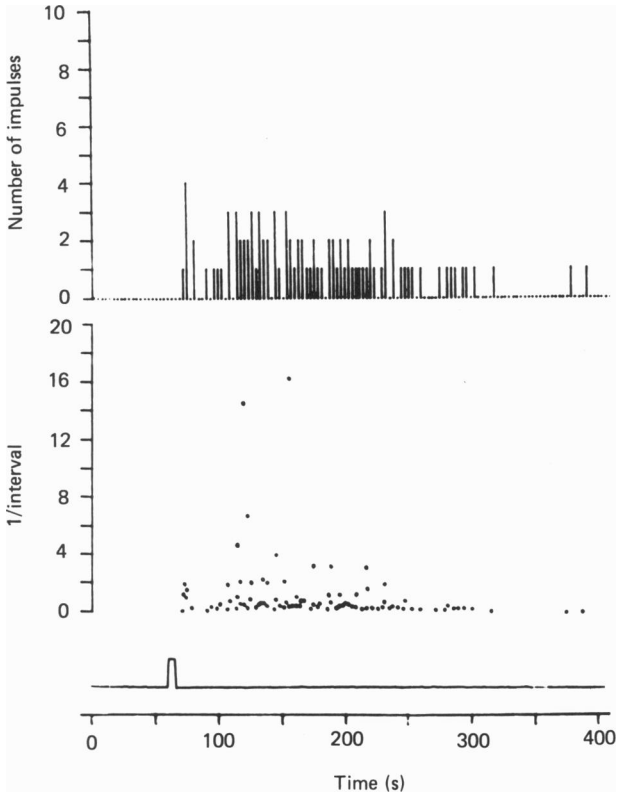

Fig. 2. Discharges of a C polymodal nociceptor to intra-arterial injection of 0·2 μg bradykinin. Ordinate: number of discharges each 3 s period (upper trace) and interval between discharge (instantaneous frequency, lower trace).

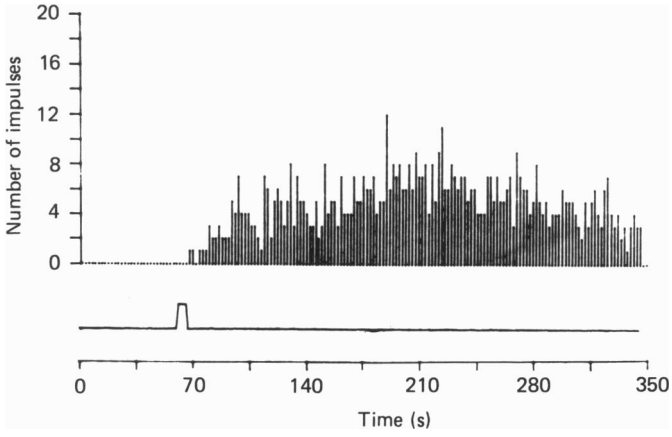

Fig. 3. Discharges of a C polymodal nociceptor evoked by chemical irritant. The receptive field was touched with cotton-wool soaked in xylene at time of the mark below graph. Number of discharges each 2 s.

ceptors to different stimuli. In these experiments identical stimuli and sequences were used (0·2 μg test doses of bradykinin and heating from 30 to 54 °C in eight steps of 17 s each). After assessing the mechanical threshold, the first bradykinin dose was injected, followed by a heating trial and a second bradykinin injection. The time interval between the heat and bradykinin test was at least 15 min; mechanical threshold was checked 7–10 min before and after these tests. Under these conditions the background activity induced by noxious heat ceased by the time of chemical stimulation and there was no difference in the number of discharges elicited by the first and the second bradykinin trials, i.e. before and after the heat trial ($53 \pm 14{\cdot}9$ and $50{\cdot}6 \pm 12{\cdot}8$ impulses, mean ± S.E., respectively). There was no demonstrable change in mechanical threshold after the bradykinin and heating trials. Following the control tests, the response to 20 μg of capsaicin was examined. Then, 5 min later, 5 × 200 μg of capsaicin was injected intra-arterially to produce a mild desensitization.

TABLE 2. Capsaicin desensitization of polymodal nociceptors

Stimulus	Mean discharge (mean ± S.E., $n = 12$, 95% confidence interval for median)					
	Control		After 2 × 200 μg capsaicin		After 5 × 200 plus 5 × 600 μg capsaicin	
48 °C	8·8	2·4 (3–10)	3·6	0·8* (0–7)	2·5	1·0* (0–4)
51 °C	10·5	2·2 (5–17)	6·8	1·8** (0–11)	4·4	1·6*** (0–9)
54 °C	21·7	5·4 (13–27)	14·9	5·2*** (0–20)	11·0	4·2*** (0–14)
Bradykinin 0·2 μg, intra-arterially	50·6	12·8 (14–104)	24·4	15·4* (0–24)	11·6	4·5*** (0–30)

Statistically significant differences from control values (Wilcoxon matched-pairs signed-rank test) are indicated as follows: * $P < 0{\cdot}05$, ** $P < 0{\cdot}02$, *** $P < 0{\cdot}01$.

15 min after this capsaicin treatment, the control sequence of mechanical, chemical and thermal stimulation was repeated. Later an additional 5 × 600 μg was given to produce a more marked desensitization and a sequence of stimulation was repeated.

The number of impulses elicited by noxious heating and bradykinin consistently decreased after the capsaicin desensitizations, with the second treatment producing a greater effect than the first (Table 2). Three units following the first desensitizations and four following the second did not respond to heating (54 °C) and/or to pressure (40 g/mm²). Bradykinin (0·2 μg) was no longer effective for four units after the first capsaicin desensitization and altogether for five units after the second. Thus, there was no preferential abolition of the effect of bradykinin, heating or pressure in terms of the number of units affected. On the other hand, for a given unit changes in responses to these three kinds of stimuli did not follow the same course. Only one unit became unresponsive to all three forms of stimuli. Rank orders of desensitization were determined on the basis of threshold differences (heat and pressure) and decrement in percentage of discharges induced by bradykinin compared to control

values. Rank correlations were positive but not statistically significant ($r \leqslant 0{\cdot}516$).

The desensitization (blocking) was relatively long lasting; suprathreshold mechanical pressure (40 g/mm^2) 60–90 min after the capsaicin treatment did not excite three units which at the time of the test of mechanical stimuli still responded to either bradykinin or noxious heating.

Three units desensitized by capsaicin were tested by topically applied xylene and exhibited markedly diminished responses. In contrast to the usual responses to xylene (e.g. Fig. 3), from these three units only one, two and nineteen impulses were evoked by topical xylene.

Other receptors

The responsiveness to such chemical stimulation was tested on fifty-three sensory units other than polymodal nociceptors. Three of these units responded to innocuous heating or cooling; their chemical sensitivity has described previously (Szolcsányi, 1983). The remaining fifty units were characterized according to the criteria set down by Burgess & Perl (1967) and Perl & Burgess (1973). The minimum pressure thresholds were 7·7–17 g/mm^2 for high-threshold C mechanoreceptors, 0·9–4·2 g/mm^2 for low-threshold C mechanoreceptors and 16–40 g/mm^2 for high-threshold Aδ mechanoreceptors. Even though a special search was made, no slowly adapting A-fibre mechanoreceptors could be found on the rabbit ear; this agrees with findings reported by others (Brown, Iggo & Miller, 1967). Table 1 illustrates the highly selective action of capsaicin and low doses of bradykinin. Six different types of low- and high-threshold mechanoreceptors (comprising all known varieties of mechanoreceptors described for the rabbit ear) were unresponsive to ten- to hundredfold larger doses of capsaicin than that which routinely excited polymodal nociceptors. One low-threshold C mechanoreceptor unit was also tested by intra-arterial injection of 1 mg capsaicin, and another by injection of 100 μg capsaicin under the receptive field; neither was excited and the threshold for pressure remained unchanged at $< 0{\cdot}9$ g/mm^2.

The small dose of bradykinin (0·2 μg) that consistently activated polymodal nociceptors did not stimulate any other type of sensory receptor tested (Table 1). A tenfold-larger dose (2 μg) excited one low-threshold and one high-threshold C mechanoreceptor unit. This suggests that bradykinin has selective action only in low concentrations.

Xylene was repeatedly applied topically to two low-threshold C mechanoreceptor units with receptive fields on the inner side of the ear that was not reached by the intra-arterial injections. No responses were observed, and after the second or third application the units became unresponsive to mechanical stimuli. Two high-threshold C mechanoreceptor units did not respond to one test with xylene; their mechanical responsiveness was not rechecked.

C-fibre responses after U.V. irradiation of the skin

Two of the eighteen C-fibre units studied in the U.V. irradiation protocol were not excited by noxious heating to 60 °C; one responded to mechanical contact with 0·9 g/mm^2 and the other had a mechanical threshold of 40 g/mm^2. They were characterized as low- and high-threshold C mechanoreceptors, respectively. The

remaining sixteen units that responded to noxious heat in the range 45–54 °C had a conduction velocity of 0·96 ± 0·18 m/s (S.D.) and mechanical thresholds that were elevated compared to the typical low-threshold types. These sixteen were also activated by chemical means (i.e. injection of bradykinin or topical application of xylene) and were therefore classified as C polymodal nociceptors. Except for two polymodal nociceptors whose discharges had been isolated before the irradiation

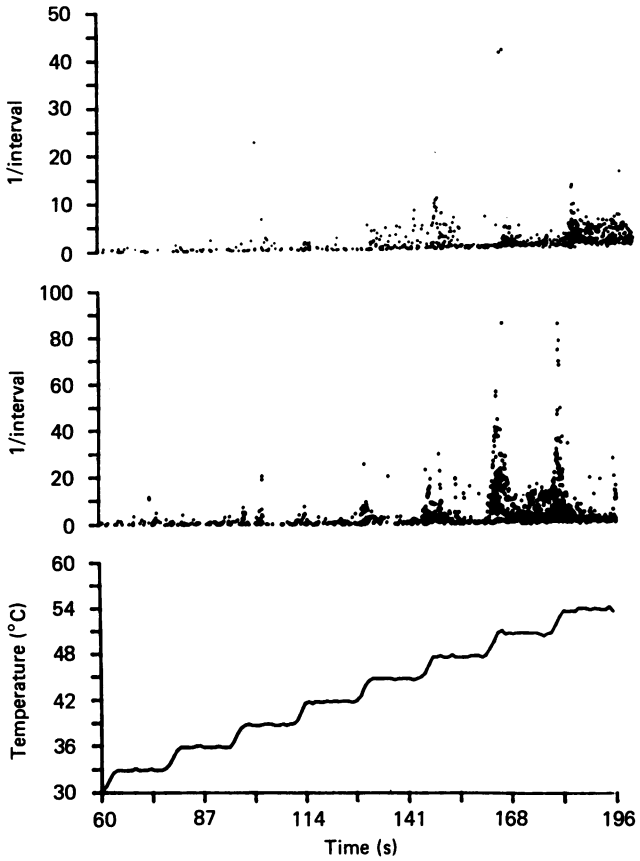

Fig. 4. Interval between discharges (instantaneous frequency) of discharges of C-fibre polymodal nociceptors to heating of the receptive field. Upper plot, twelve units not subject to U.V. irradiation; middle plot, twelve units previously irradiated with U.V. light several hours before the recording.

began, all other units studied were isolated 2·5–5 h after the U.V. irradiation. One Aδ mechanoreceptor (conduction velocity, c.v. 22 m/s, mechanical threshold 17 g/mm^2) was also studied in this series.

All thirteen polymodel nociceptors with receptive fields on the irradiated dorsum of the ear exhibited a low-frequency ongoing activity, something almost never encountered in such units from uninjured and unstimulated skin. The rate of discharges before test stimulation was 6·64 ± 4·18 impulses/min (S.D.). Background discharge was not seen in one polymodal nociceptor with a receptive field in the unexposed side of the ear and in the three other types of sensory receptors. Two

polymodal nociceptors in different preparations were isolated before the U.V. irradiation to permit study of the evolution of the ongoing activity; the initial background discharge by these units was observed approximately 30 min after the beginning of irradiation.

The effect of chemical and noxious heating was analysed on eleven polymodal nociceptors of the dorsum of the ear, isolated after U.V. irradiation. The number of discharges evoked by noxious heating varied considerably in this group. In response to heating to 48, 51 and 54 °C, the mean discharge (95% confidence interval for median) were: 15·8 (10–31), 32·9 (10–104) and 34·0 (14–59) impulses, respectively.

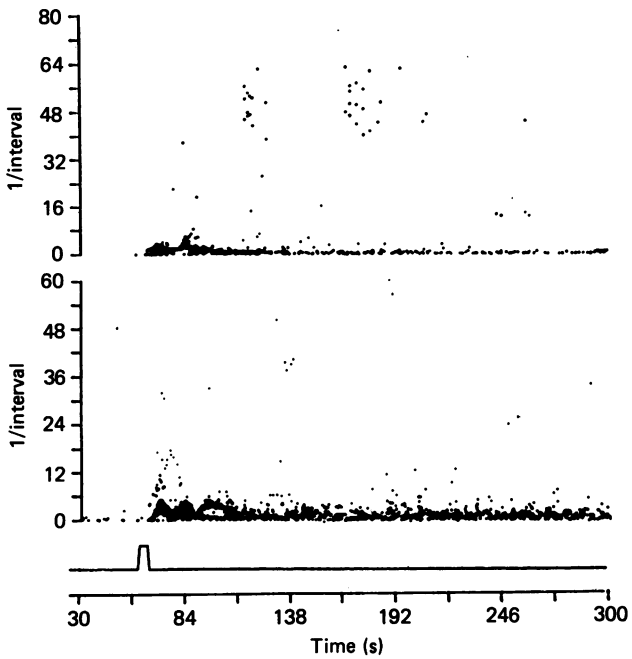

Fig. 5. Interval between discharges (instantaneous frequency) of polymodal nociceptors to intra-arterial injection of bradykinin. Upper plot, stimulated responses evoked by 0·2 μg bradykinin on twelve units not exposed to U.V. The lower plot, responses evoked for an identical dose of bradykinin for nine C polymodal nociceptors from ears irradiated with U.V. light for 4 h, 2·5–5 h prior to these observations.

These values were much higher than for corresponding measures from untreated polymodal nociceptors ($n = 15$): 5·9 (4–9), 10·5 (4–15) and 26·2 (13–52). According to the Wilcoxon rank sum test, the increased discharge in the irradiated group is significant ($P < 0·02$) at the temperature steps of 48 and 51 °C, but not at 54 °C. The latter is possibly explicable by a fatigue-like adaptation commonly seen in strongly excited polymodal nociceptors; note the fall from the high peak value in the middle plot of Fig. 4 at the 54 °C step.

The response of polymodal nociceptors to bradykinin (0·2 μg) after U.V. irradiation was increased both in the duration of activity and the number of discharges as illustrated in Fig. 5. (Note Fig. 5 compares the total activity for twelve untreated polymodal units to nine after U.V. exposure.) However, the latency of the response

of the irradiated group to intra-arterial bradykinin (9·3 ± 1·2 s mean ± S.E., $n = 9$) remained similar to that of control units. Xylene applied to the receptive field of five units evoked an average of 926 ± 107 mean ± S.E. discharges in 5 min. This value is greater than that found for untreated polymodal nociceptors, but data suitable for quantitative comparison were not collected.

DISCUSSION

Excitation of afferent units of the intact skin by chemical means

The observations just presented indicate that C polymodal nociceptors are the only cutaneous sense organs commonly encountered in the innervation of the rabbit's ear that are effectively excited by small intra-arterial doses of bradykinin and capsaicin. The same small doses of bradykinin and capsaicin injected intra-arterially into the rabbit ear elicit nociceptive reflexes (Juan & Lembeck, 1974; Juan, Lembeck, Seewann & Hack, 1980). Together these two facts offer still another confirmation that nociceptive sense organs by themselves are sufficient to initiate a pain-like reaction. In contrast, serotonin (5-HT), an agent which has been reported to excite sense organs other than nociceptors (Fjällbrandt & Iggo, 1961), failed to excite polymodal nociceptors in doses up to 20 μg; this quantity of serotonin given intra-arterially to the rabbit ear does not elicit nociceptive reflexes (Juan & Lembeck, 1974).

Capsaicin may be an agent capable of selectively exciting C-fibre polymodal nociceptors over a substantial range of doses (100 ×); however, additional data are needed to confirm this. Experiments will be necessary to test capsaicin's action on slowly adapting A mechanoreceptors, which are not present in the rabbit ear (Brown *et al.* 1967), on specific warm fibres, which were not encountered in the present survey, and on subcutaneous sense organs, which were not studied.

Work by others has shown that microgram quantities of bradykinin excite autonomic neurones and several types of sensory receptors (Fjällbrandt & Iggo, 1961; Lewis & Reit, 1965; Beck & Handwerker, 1974); the present analysis confirms this for cutaneous sensory units although C polymodal nociceptors may have relatively low thresholds for this agent. The past and present observation on the effects of bradykinin appear consonant with reports that low doses of bradykinin activated only nocireceptive dorsal horn neurones of the cat, while higher doses also excited units driven by innocuous mechanical stimuli (Belcher, 1979). It is worth noting that bradykinin is released in response to tissue injury in nanogram quantities, and that the composite kinin-forming ability of rabbit's blood is equivalent to 6–10 μg/ml bradykinin (Garcia Leme, 1978). Thus, very low bradykinin concentrations, near the threshold for intra-arterial excitation of polymodal nociceptors, probably approach *in vivo* release quantities in severe inflammatory conditions (Bonta & de Vox, 1967).

Capsaicin desensitization

The response of C polymodal nociceptors to thermal, mechanical and chemical stimuli was suppressed or abolished by repeated high doses of capsaicin. Therefore capsaicin desensitization represents one type of nociceptor's decreased responsiveness to its ordinary (adequate) sources of stimulation. It is interesting that the changes

in responsiveness to the kinds of adequate stimuli did not run in parallel. Since in all but one unit, even at the time of maximal desensitization, responses could still be evoked by at least one kind of stimulus, it seems unlikely that the suppression originates from a block of the conduction by the afferent fibre proximal to the receptive terminals (Petsche, Fleischer, Lembeck & Handwerker, 1983; Handwerker *et al.* 1984; Lynn *et al.* 1984). Furthermore, the dissociation in responsiveness suggests that impulse-generating mechanisms for polymodal nociceptors for mechanical, thermal or chemical stimuli may be independent to some extent, although all are susceptible to capsaicin blocking. Under similar experimental conditions cold receptors are not desensitized (Szolcsányi, 1983).

Taken together, the various past (Petsche *et al.* 1983; Szolcsányi, 1983, 1985; Handwerker *et al.* 1984; Lynn *et al.* 1984) and present findings on capsaicin action indicate that in adult animals this agent is not a general sensory C-fibre stimulant or neurotoxin; it clearly has a selective effectiveness on certain C-fibre sensory neurones. On the other hand, in the immature nervous system of neonatal rats parenteral capsaicin has a broader target, and less specific destruction of afferent C- and Aδ-fibre neurones occurs (Handwerker *et al.* 1984; Lynn *et al.* 1984; Welk *et al.* 1984).

Effect of U.V. erythema on polymodal nociceptors

After exposure to inflammatory amounts of U.V. radiation, polymodal nociceptors develop background activity and substantially increased responses to bradykinin and noxious heat; however, a lowered heat threshold was not as obvious during the immediate post-radiation period. Sensitization of polymodal nociceptors by noxious heat (Bessou & Perl, 1969; King, Gallant, Myerson & Perl, 1976; Kumazawa & Perl, 1977; Lynn, 1979; Shea & Perl, 1985), by mechanical stimulation of the skin (Fitzgerald, 1979), or by infusion of prostaglandins of the E series (Chahl & Iggo, 1977) has been well documented. Sensitization of polymodal nociceptors cannot be attributed to cutaneous vasodilatation or normal blood constituents (King *et al.* 1976). There is evidence for release of prostaglandins, histamine and serotonin, and for degranulation of cutaneous mast cells after U.V. irradiation of the guinea-pig, rat or human skin (Logan & Wilhelm, 1966). It is likely that this kind of injury releases a similar combination of chemical substances from the rabbit skin, although there is less information on this species (Swingle, Rancik & Kvam, 1979). Thus, the present analyses agree with postulates that sensitization of polymodal nociceptors to thermal, chemical or mechanical stimuli appears to be the result of a combined facilitative action by several chemical agents produced in conditions causing inflammation (King *et al.* 1976).

The effect of U.V. irradiation on other types of sensory nerve endings needs further investigation. In the present experiments, after U.V. exposure background activity was not seen in units other than polymodal nociceptors and a few D-hair receptors situated near the pulsatile vessels. Similar ongoing discharges from D-hair units has been reported in normal animals (Perl, 1968) and in the present experiments could be abolished by small shifts of the skin's position. Therefore, only the development of ongoing discharge by polymodal nociceptors could be attributed to changes resulting from the U.V. irradiation. In any case, the part played by edema and other

physical rearrangements must be considered from adequately evaluating and interpreting changes in background activity of sensory units from inflammed tissues.

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